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IMMUNOLOGIC INTERRELATIONSHIPS OF COLIFORM  
HEAT-LABILE AND HEAT-STABLE ENTEROTOXINS

Final Report

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)  Investigations conducted during the four year period (1977 to 1981) of support by the USAMRDC have accomplished the following. (1) The enterotoxigenicity of certain coliform species which contaminate the small bowel in different diarrheal disorders has been established. (2) Immunization regimens have been developed which provide protection against enterotoxigenic strains of <i>E. coli</i> in experimental animals.		

SUMMARY

These investigations (a) established the fact that species of coliform bacteria other than ETEC strains of *E. coli* elaborate enterotoxins which alter gastrointestinal physiology, and (b) showed that immunization with either *E. coli* (ETEC) LT or ST toxin arouses an antitoxin response in experimental animals which protects against challenge with viable ETEC strains which produce the toxin used for immunization. Information regarding optimal immunization routes and schedules, antitoxin responses, and toxoiding of these immunogens has also been obtained. This information is being used to facilitate current work in this laboratory to develop a practical vaccine which will provide protection against diarrheal disease caused by all types of ETEC strains in humans. In view of the well-recognized incapacitating effect of acute diarrheal disease among military personnel serving in the tropics, the successful development of the vaccine should have major health implications for the military.

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FOREWARD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

## INTRODUCTION

This final report summarizes research activities conducted in this laboratory during the four year period between 1977 and 1981 that were supported by the USAMRDC and The Office of Naval Research. Since the results of all but one of the investigations conducted have been described in full detail in previously submitted annual reports, as well as in the publications listed, this report will confine itself to a brief overview of these investigations. They may be divided into two general categories as follows.

(1) Characterization of coliform enterotoxins. An assay was developed which detects, by means of in vivo marker perfusion in the rat jejunum, the presence of heat-labile (LT) or heat-stable (ST) enterotoxins produced by coliform strains other than enterotoxigenic strains of *Escherichia coli* (ETEC) (10). The toxins detected usually do not stimulate the conventional assay systems such as the Y1 adrenal cell assay for LT and the suckling mouse assay for ST. The strains examined included *Klebsiella pneumoniae*, *Enterobacter cloacae* and enteropathogenic strains of *E. coli* (EPEC) which were isolated from the gastrointestinal tract of persons with either acute or chronic diarrhea. The biologic properties of these toxins including their physical properties, their effect on gastrointestinal physiology, and their immunologic properties were delineated in a series of studies.

In summary, strains of non-ETEC coliform bacteria isolated from the jejunum of persons with tropical sprue were shown to elaborate toxins whereas those isolated from the jejunum of individuals with the blind loop syndrome or from the stool of normal persons do not (5,7). These toxins affect water and glucose transport (3,6,7). The heat-labile toxins elaborated by these strains were shown to have immunological cross-reactivity with similarly prepared toxins derived from ETEC strains (1,2). Strains of *E. coli* isolated from nursery outbreaks of infantile diarrhea, which were negative in conventional assays for ETEC toxins, were also shown to elaborate toxins by means of this assay (4) and an immunological relationship between these toxins and those elaborated by ETEC strains was established (16).

(2) Development of a program of immunological protection against diarrheal disease due to intestinal colonization by enterotoxigenic strains of *Escherichia coli*.

Preliminary studies conducted in 1978 showed that immunization of rats with an impure form (polymyxin-release) of the *E. coli* (ETEC) heat-labile (LT) toxin yields protection against challenge with either this toxin or viable ETEC LT-producing strains but not against the heat-stable toxin (ST), or viable strains which produce this toxin form (8). Subsequent investigations conducted in 1979 and 1980 delineated the influence of the immunization route, schedule and dosage of this immunogen on immediate and extended protection in this animal model (13,14,17). The validity of these observations, which were conducted using ligated ileal loops as the challenge technique to the intact gut, was established by studies which demonstrated protection in immunized gnotobiotic rats which were challenged by intestinal colonization with ETEC strains (15).

Following publication in 1980 of the methods to produce pure, homogeneous LT holotoxin and its B subunit, we made and used these materials to immunize rats. The LT holotoxin was found to be a more effective immunogen on a molar basis than the B subunit (18). Immunization with the LT holotoxin was shown to arouse an antitoxin response within the serum and/or intestinal mucosa (depending on the route of immunization), as detected by enzyme-linked immunosorbent assay (21), which provides protection against heterologous serotypes of viable LT-producing strains (20). Following publication in 1981 of methods to produce the ST toxin, we made it and immunized rats with the ST toxin coupled to a large molecular weight carrier; this immunization was shown to provide protection against this toxin and viable ST-producing strains but not against LT-producing strains (21).

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